On page 6, delete the third full paragraph under the "Brief Description of the Drawings," and replace this paragraph with the following:

Figures 3A-B present amino acid sequences (SEQ ID NOS 2-6) of representative microbialβ-glucuronidases.

On page 6, delete the fourth full paragraph under the "Brief Description of the Drawings," and replace this paragraph with the following:

Figures 4A-J present DNA sequences (SEQ ID NOS 7-14) of representative microbial β-glucuronidases.

On page 6, delete the fifth full paragraph under the "Brief Description of the Drawings," and replace this paragraph with the following:

Figures 5A-C present amino acid alignments of *Staphylococcus* GUS (SGUS) (SEQ ID NO: 15) *E. coli* GUS (EGUS) (SEQ ID NO: 17) and human GUS (HGUS) (SEQ ID NO: 16) (5A). Microbial GUSes (5B) and nucleotide sequence alignments (SEQ ID NOS 18-23 and 24-26) of *Staphylococcus, Salmonella,* and *Pseudomonas* β-glucuronidases.

On page 7, delete the fourth full paragraph, and replace this paragraph with the following:

Figures 13A-C present a DNA sequence of *Staphylococcus* GUS (SEQ ID NO: 27 and 28) that is codon-optimized for production in *E. coli*.

On page 7, delete the seventh full paragraph, and replace this paragraph with the following:

Figure 16 is a DNA sequence of a *Salmonella* gene β--glucuronidase (SEQ ID NO: 113).

On page 7, delete the eighth full paragraph, and replace this paragraph with the following:

Figure 17 is an amino acid sequence of a *Salmonella* gene β--glucuronidase translated from the DNA sequence (SEQ ID NO: 114).

On page 7, delete the ninth full paragraph, and replace this paragraph with the following:

Figure 18A-C presents an alignment of amino acids of three β--glucuronidase gene products: Staph (*Staphylococcus*) (SEQ ID NO: 18), E. coli (SEQ ID NO: 23), Sal (a *Salmonella*) (SEQ ID NO: 114).

On page 7, delete the tenth full paragraph, and replace this paragraph with the following:

Figure 19A-G presents an alignment of nucleotides of three β--glucuronidases; Staph (*Staphylococcus*) (SEQ ID NO: 115), E. coli (SEQ ID NO: 116), Sal (*Salmonella*) (SEQ ID NO: 113).

On page 8, delete the first full paragraph, and replace this paragraph with the following:

As used herein, a "secreted form of a microbial β-glucuronidase" refers to a microbial β-glucuronidase that is capable of being localized to an extracellular environment of a cell, including extracellular fluids, periplasm, or is membrane bound on the external face of a cell but is not an integral membrane protein. Some of the protein may be found intracellularly. The amino acid and nucleotide sequences of exemplary secreted β-glucuronidases are presented in Figures 1 and 16 and SEQ ID Nos.: 1, and 113. Secreted microbial GUS also encompasses variants of β-glucuronidase. A variant may be a portion of the secreted β-glucuronidase and/or have amino acid substitutions, insertions, and deletions, either found naturally as a polymorphic allele or constructed. A variant may also be a fusion of all or part of GUS with another protein.

On page 12, delete the third full paragraph, and replace this paragraph with the following:

A DNA sequence of the GUS gene contained in the insert of pRAJa17.1 is presented in Figure 1 and as SEQ ID No: 1. A schematic of the insert is presented in Figure 2. The β-glucuronidase gene contained in the insert is identified by similarity of the predicted amino acid sequence of an open reading frame to the *E. coli* and human β-glucuronidase amino acid sequences (Figure 5A). Overall, *Staphylococcus* β--glucuronidase has approximately 47-49% amino acid identity to *E. coli* GUS and to human GUS. An open reading frame of *Staphylococcus* GUS is 1854 bases, which would result in a protein that is 618 amino acids in length. The first methionine codon, however, is unlikely to encode the initiator methionine. Rather the second methionine codon is most likely the initiator methionine. Such a translated product is 602 amino acids long and is the sequence presented in Figures 3A-B and 4A-I. The assignment of the initiator methionine is based upon a consensus Shine-Dalgarno sequence found upstream of the second Met, but not the first Met, and alignment of the

Staphylococcus, human, and *E. coli* GUS amino acid sequences. Furthermore, as shown herein, *Staphylococcus* GUS gene lacking sequence encoding the 16 amino acids is expressed in *E. coli* transfectants. In addition, the 16 amino acids (Met-Leu-Ile-Ile-Thr-Cys-Asn-His-Leu-His-Leu-Lys-Arg-Ser-Ala-Ile) SEQ ID No. 29 are not a canonical signal peptide sequence.

On page 13, delete the third full paragraph, and replace this paragraph with the following:

The DNA sequences of GUS genes from *Staphylococcus homini*, *Staphylococcus warneri*, *Thermotoga maritima* (TIGR *Thermotoga* database), *Enterobacter*, *Salmonella*, and *Pseudomonas* are presented in Figures 4A-J and SEQ ID Nos. 7-14. Predicted amino acid sequences are shown in Figures 3A-B and SEQ ID Nos. 2-6. The amino acid sequences are shown in alignment in Figures 5A-C. The signature peptide sequences for glycosyl hydrolases (Henrissat, *Biochem Soc Trans 26:*153, 1998; Henrissat B *et al., FEBS Lett 27:*425, 1998) are located from amino acids 333 to 358 and from amino acids 406 to 420 (*Staphylococcus* numbering in Figures 3A and 5B). The catalytic nucleophile is Glu 344 (*Staphylococcus* numbering) (Wong *et al., J. Biol Chem. 18*: 34057, 1998). Within these two signature regions, 17/26 and 8/15 residues are identical across the six presented sequences. At the non-identical positions, most of the sequences share an identical residue. Thus, the sequences are highly conserved in these regions (identity between *Staphylococcus* and each other GUS gene ranges from 65% to 100% in signature 1 and from 73% to 100% in signature 2) (*see* Figure 5B). In contrast, between *Staphylococcus* and β-galactosidase, another glycosyl hydrolase that has signature sequences, identity is 46% in signature 1 and 73% in signature 2.

On page 18, delete the second full paragraph, and replace this paragraph with the following:

In other embodiments, variants may exhibit glucuronide binding activity without enzymatic activity or be directed to other cellular compartments, such as membrane or cytoplasm. Membrane-spanning amino acid sequences are generally hydrophobic and many examples of such sequences are well-known. These sequences may be spliced onto microbial secreted GUS by a variety of methods including conventional recombinant DNA techniques. Similarly, sequences that direct proteins to cytoplasm (*e.g.*, Lys-Asp-Glu-Leu (SEQ ID NO: 30)) may be added to the reference GUS, typically by recombinant DNA techniques.

On page 18, delete the third full paragraph, and replace this paragraph with the following:

In other embodiments, a fusion protein comprising GUS may be constructed from the nucleic acid molecule encoding microbial and another nucleic acid molecule. As will be appreciated, the fusion partner gene may contribute, within certain embodiments, a coding region. In preferred embodiments, microbial GUS is fused to avidin, streptavidin or an antibody. Thus, it may be desirable to use only the catalytic site of GUS (*e.g.*, amino acids 415-508 reference to *Staphylococcus* sequence). The choice of the fusion partner depends in part upon the desired application. The fusion partner may be used to alter specificity of GUS, provide a reporter function, provide a tag sequence for identification or purification protocols, and the like. The reporter or tag can be any protein that allows convenient and sensitive measurement or facilitates isolation of the gene product and does not interfere with the function of GUS. For example, green fluorescent protein and β-galactosidase are readily available as DNA sequences. A peptide tag is a short sequence, usually derived from a native protein, which is recognized by an antibody or other molecule. Peptide tags include FLAG®, Glu-Glu tag (Chiron Corp., Emeryville, CA), KT3 tag (Chiron Corp.), T7 gene 10 tag (Invitrogen, La Jolla, CA), T7 major capsid protein tag (Novagen, Madison, WI), His_6 (SEQ ID NO: 117)

(hexa-His), and HSV tag (Novagen). Besides tags, other types of proteins or peptides, such as glutathione-S-transferase may be used.

On page 19, delete the first full paragraph, and replace this paragraph with the following:

In other aspects of the present invention, isolated microbial glucuronidase proteins are provided. In one embodiment, GUS protein is expressed as a hexa-His (SEQ ID NO: 117) fusion protein and isolated by metal-containing chromatography, such as nickel-coupled beads. Briefly, a sequence encoding His_6 (SEQ ID NO: 117) is linked to a DNA sequence encoding a GUS. Although the His_6 (SEQ ID NO: 117) sequence can be positioned anywhere in the molecule, preferably it is linked at the 3' end immediately preceding the termination codon. The His-GUS fusion may be constructed by any of a variety of methods. A convenient method is amplification of the GUS gene using a downstream primer that contains the codons for His_6 (SEQ ID NO: 117).

On page 42, delete the second full paragraph, and replace this paragraph with the following:

The presence of GUS genes is established by amplification using degenerate oligonucleotides derived from a conserved region of the GUS gene. A pair of oligonucleotides is designed using an alignment of *E. coli* gusA and human GUS sequences. The primer T3-GUS-2F covers *E. coli* GUS amino acids 163-168 (DFFNYA)(SEQ ID NO: 31), while T7-GUS-5B covers the complementary sequence to amino acids 549-553 (WNFAD)(SEQ ID NO: 32). The full length of *E. coli* GUS is 603 amino acids. As shown in Table 1, amplification is not always successful, likely due to mismatching of the primers with template. Thus, a negative amplification does not necessarily signify that the microorganism lacks a GUS gene.

On page 49 delete the last paragraph under the heading "Example 6, Construction of a Codon Optimized Secreted β-Glucuronidase," and replace this paragraph with the following:

The *Staphylococcus* GUS gene is codon-optimized for expression in *E. coli* and in rice. Codon frequencies for each codon are determined by back translation using ecohigh codons for highly expressed genes of enteric bacteria. These ecohigh codon usages are available from GCG. The most frequently used codon for each amino acid is then chosen for synthesis. In addition, the polyadenylation signal, AATAAA, splice consensus sequences, ATTTA AGGT, and restriction sites that are found in polylinkers are eliminated. Other changes may be made to reduce potential secondary structure. To facilitate cloning in various vectors, four different 5' ends are synthesized: the first, called AO (GT CGA CCC ATG GTA GAT CTG *ACT AGT* CTG TAC CCG) (SEQ ID NO: 51) uses a sequence comprising an *Nco* I (underlined), *Bgl* II (double underlined), and *Spe* I (italicized) sites. The Leu (CTG) codon is at amino acid 2 in Figures 3A-B. The second variant, called AI (*GTC GAC* AGG AGT GCT ATC ATG CTG TAC CCG) (SEQ ID NO: 52) adds the native Shine/Dalgarno sequence 5' of the initiator Met (ATG) codon; the third, called AII, (*GTC GAC* AGG AGT GCT ACC ATG GTG TAC CCG) (SEQ ID NO: 53) adds a modified Shine/Dalgarno sequence 5' of the initiator Met codon such that a *Nco* I site is added; the fourth one, called AIII (*GTC GAC* AGG AGT GCT ACC ATG GTA GAT CTG TAC CCG) (SEQ ID NO:54) adds a modified Shine/Dalgarno sequence 5' of the Leu (CTG) codon (residue 2) and *Nco* I and *Bgl* II sites. All of these new 5' sequences contain a *Sal* I site at the extreme 5' end to facilitate construction and cloning. In certain embodiments, to facilitate protein purification, a sequence comprising a *Nhe* I, *Pml* I, and *Bst*E II sites (underlined) and encoding hexa-His amino acids joined at the 3' (COOH-terminus) of the gene.

GCTAGCCATCACCATCACCATCACGTGTGAATTGGTGACCG (SEQ ID NO: 55)

SerSerHisHisHisHisHisVal * (SEQ ID NO: 56)

On page 50 **Table 3**, insert numbers in column four as follows:

Table 3

Oligonucleotide	Size	Sequence	SEQ ID NO
$gusA^{Stp}$ A-1-80T	80	TCGACCCATGGTAGATCTGACTAGTCTGTAC CCGATCAACACCGAGACCCGTGGCGTCTTC GACCTCAATGGCGTCTGGA	57
$gusA^{Stp}$ A-121-200B	80	GGATTTCCTTGGTCACGCCAATGTCATTGTA ACTGCTTGGGACGGCCATACTAATAGTGTC GGTCAGCTTGCTTTCGTAC	58
$gusA^{Stp}$ A-161-240T	80	CCAAGCAGTTACAATGACATTGGCGTGACC AAGGAAATCCGCAACCATATCGGATATGTC TGGTACGAACGTGAGTTCAC	59
$gusA^{Stp}$ A-201-280B	80	GCGGAGCACGATACGCTGATCCTTCAGATA GGCCGGCACCGTGAACTCACGTTCGTACCA GACATATCCGATATGGTTGC	60
$gusA^{Stp}$ A-241-320T	80	GGTGCCGGCCTATCTGAAGGATCAGCGTAT CGTGCTCCGCTTCGGCTCTGCAACTCACAA AGCAATTGTCTATGTCAATG	61
$gusA^{Stp}$ A-281-360B	80	AATGGCAGGAATCCGCCCTTGTGCTCCACG ACCAGCTCACCATTGACATAGACAATTGCT TTGTGAGTTGCAGAGCCGAA	62
$gusA^{Stp}$ A-321-400T	80	GTGAGCTGGTCGTGGAGCACAAGGGCGGAT TCCTGCCATTCGAAGCGGAAATCAACAACT CGCTGCGTGATGGCATGAAT	63
$gusA^{Stp}$ A-361-460B	100	GTACAGCCCCACCGGTAGGGTGCTATCGTC GAGGATGTTGTCCACGGCGACGGTGACGCG ATTCATGCCATCACGCAGCGAGTTGTTGATT TCCGCTTCG	64
$gusA^{Stp}$ A-401-456T	56	CGCGTCACCGTCGCCGTGGACAACATCCTC GACGATAGCACCCTACCGGTGGGGCT	65
$gusA^{Stp}$ A-41-120B	80	CACTTCTCTTCCAGTCCTTTCCCGTAGTCCA GCTTGAAGTTCCAGACGCCATTGAGGTCGA AGACGCCACGGGTCTCGGT	66
$gusA^{Stp}$ A-6-40B	35	TTGATCGGGTACAGACTAGTCAGATCTACC ATGGG	67
$gusA^{Stp}$ A-81-160T	80	ACTTCAAGCTGGACTACGGGAAAGGACTGG AAGAGAAGTGGTACGAAAGCAAGCTGACC GACACTATTAGTATGGCCGTC	68
$gusA^{Stp}$ B-1-80T	80	GTACAGCGAGCGCCACGAAGAGGGCCTCG GAAAAGTCATTCGTAACAAGCCGAACTTCG ACTTCTTCAACTATGCAGGCC	69

Oligonucleotide	Size	Sequence	SEQ ID NO
$gusA^{Stp}$ B-121-200B	80	CTTTGCCTTGAAAGTCCACCGTATAGGTCAC AGTCCCGGTTGGGCCATTGAAGTCGGTCAC AACCGAGATGTCCTCGACG	70
$gusA^{Stp}$ B-161-240T	80	ACCGGGACTGTGACCTATACGGTGGACTTT CAAGGCAAAGCCGAGACCGTGAAAGTGTC GGTCGTGGATGAGGAAGGCAA	71
$gusA^{Stp}$ B-201-280B	80	CTCCACGTTACCGCTCAGGCCCTCGGTGCTT GCGACCACTTTGCCTTCCTCATCCACGACCG ACACTTTCACGGTCTCGG	72
$gusA^{Stp}$ B-241-320T	80	AGTGGTCGCAAGCACCGAGGGCCTGAGCGG TAACGTGGAGATTCCGAATGTCATCCTCTG GGAACCACTGAACACGTATC	73
$gusA^{Stp}$ B-281-360B	80	GTCAGTCCGTCGTTCACCAGTTCCACTTTGA TCTGGTAGAGATACGTGTTCAGTGGTTCCCA GAGGATGACATTCGGAAT	74
$gusA^{Stp}$ B-321-400T	80	TCTACCAGATCAAAGTGGAACTGGTGAACG ACGGACTGACCATCGATGTCTATGAAGAGC CGTTCGGCGTGCGGACCGTG	75
$gusA^{Stp}$ B-361-440B	80	ACGGTTTGTTGTTGATGAGGAACTTGCCGTC GTTGACTTCCACGGTCCGCACGCCGAACGG CTCTTCATAGACATCGATG	76
$gusA^{Stp}$ B-401-480T	80	GAAGTCAACGACGGCAAGTTCCTCATCAAC AACAAACCGTTCTACTTCAAGGGCTTTGGC AAACATGAGGACACTCCTAT	77
$gusA^{Stp}$ B-41-120B	80	TACGTAAACGGGGTCGTGTAGATTTTCACC GGACGGTGCAGGCCTGCATAGTTGAAGAAG TCGAAGTTCGGCTTGTTACG	78
$gusA^{Stp}$ B-441-520B	80	ATCCATCACATTGCTCGCTTCGTTAAAGCCA CGGCCGTTGATAGGAGTGTCCTCATGTTTGC CAAAGCCCTTGAAGTAGA	79
$gusA^{Stp}$ B-481-555T	75	CAACGGCCGTGGCTTTAACGAAGCGAGCAA TGTGATGGATTTCAATATCCTCAAATGGATC GGCGCCAACAGCTT	80
$gusA^{Stp}$ B-5-40B	36	AATGACTTTTCCGAGGCCCTCTTCGTGGCGC TCGCT	81
$gusA^{Stp}$ B-521-559B	39	CCGGAAGCTGTTGGCGCCGATCCATTTGAG GATATTGAA	82
$gusA^{Stp}$ B-81-160T	80	TGCACCGTCCGGTGAAAATCTACACGACCC CGTTTACGTACGTCGAGGACATCTCGGTTGT GACCGACTTCAATGGCCCA	83
$gusA^{Stp}$ C-1-80T	80	CCGGACCGCACACTATCCGTACTCTGAAGA GTTGATGCGTCTTGCGGATCGCGAGGGTCT GGTCGTGATCGACGAGACTC	84
$gusA^{Stp}$ C-121-200B	80	GTTCACGGAGAACGTCTTGATGGTGCTCAA ACGTCCGAATCTTCTCCCAGGTACTGACGC GCTCGCTGCCTTCGCCGAGT	85

Oligonucleotide	Size	Sequence	SEQ ID NO
gusA^{Stp} C-161-240T	80	ATTCGGACGTTTGAGCACCATCAAGACGTT CTCCGTGAACTGGTGTCTCGTGACAAGAAC CATCCAAGCGTCGTGATGTG	86
gusA^{Stp} C-201-280B	80	CGCGCCCTCTTCCTCAGTCGCCGCCTCGTTG GCGATGCTCCACATCACGACGCTTGGATGG TTCTTGTCACGAGACACCA	87
gusA^{Stp} C-241-320T	80	GAGCATCGCCAACGAGGCGGCGACTGAGG AAGAGGGCGCGTACGAGTACTTCAAGCCGT TGGTGGAGCTGACCAAGGAAC	88
gusA^{Stp} C-281-360B	80	ACAAACAGCACGATCGTGACCGGACGCTTC TGTGGGTCGAGTTCCTTGGTCAGCTCCACCA ACGGCTTGAAGTACTCGTA	89
gusA^{Stp} C-321-400T	80	TCGACCCACAGAAGCGTCCGGTCACGATCG TGCTGTTTGTGATGGCTACCCCGGAGACGG ACAAAGTCGCCGAACTGATT	90
gusA^{Stp} C-361-440B	80	CGAAGTACCATCCGTTATAGCGATTGAGCG CGATGACGTCAATCAGTTCGGCGACTTTGTC CGTCTCCGGGGTAGCCATC	91
gusA^{Stp} C-401-489T	89	GACGTCATCGCGCTCAATCGCTATAACGGA TGGTACTTCGATGGCGGTGATCTCGAAGCG GCCAAAGTCCATCTCCGCCAGGAATTTCA	92
gusA^{Stp} C-41-120B	80	CCCGTGGTGGCCATGAAGTTGAGGTGCACG CCAACTGCCGGAGTCTCGTCGATCACGACC AGACCCTCGCGATCCGCAAG	93
gusA^{Stp} C-441-493B	53	CGCGTGAAATTCCTGGCGGAGATGGACTTT GGCCGCTTCGAGATCACCGCCAT	94
gusA^{Stp} C-5-40B	36	ACGCATCAACTCTTCAGAGTACGGATAGTG TGCGGT	95
gusA^{Stp} C-81-160T	80	CGGCAGTTGGCGTGCACCTCAACTTCATGG CCACCACGGGACTCGGCGAAGGCAGCGAG CGCGTCAGTACCTGGGAGAAG	96
gusA^{Stp} D-1-80T	80	CGCGTGGAACAAGCGTTGCCCAGGAAAGCC GATCATGATCACTGAGTACGGCGCAGACAC CGTTGCGGGCTTTCACGACA	97
gusA^{Stp} D-121-200B	80	TCGCGAAGTCCGCGAAGTTCCACGCTTGCT CACCCACGAAGTTCTCAAACTCATCGAACA CGACGTGGTTCGCCTGGTAG	98
gusA^{Stp} D-161-240T	80	TTCGTGGGTGAGCAAGCGTGGAACTTCGCG GACTTCGCGACCTCTCAGGGCGTGATGCGC GTCCAAGGAAACAAGAAGGG	99
gusA^{Stp} D-201-280B	80	GTGCGCGGCGAGCTTCGGCTTGCGGTCACG AGTGAACACGCCCTTCTTGTTTCCTTGGACG CGCATCACGCCCTGAGAGG	100
gusA^{Stp} D-241-320T	80	CGTGTTCACTCGTGACCGCAAGCCGAAGCT CGCCGCGCACGTCTTTCGCGAGCGCTGGAC CAACATTCCAGATTTCGGCT	101

Oligonucleotide	Size	Sequence	SEQ ID NO
gusA^{Stp} D-281-369B	89	CGGTCACCAATTCACACGTGATGGTGATGG TGATGGCTAGCGTTCTTGTAGCCGAAATCTG GAATGTTGGTCCAGCGCTCGCGAAAGAC	102
gusA^{Stp} D-321-373T	53	ACAAGAACGCTAGCCATCACCATCACCATC ACGTGTGAATTGGTGACCGGGCC	103
gusA^{Stp} D-41-120B	80	TACTCGACTTGATATTCCTCGGTGAACATCA CTGGATCAATGTCGTGAAAGCCCGCAACGG TGTCTGCGCCGTACTCAGT	104
gusA^{Stp} D-5-40B	36	GATCATGATCGGCTTTCCTGGGCAACGCTTG TTCCA	105
gusA^{Stp} D-81-160T	80	TTGATCCAGTGATGTTCACCGAGGAATATC AAGTCGAGTACTACCAGGCGAACCACGTCG TGTTCGATGAGTTTGAGAAC	106

On page 55 delete the paragraph stating with "Invertase signal sequence:" and replace this paragraph with the following:

Invertase signal sequence:

ATGCTTTTGC AAGCCTTCCT TTTCCTTTTG GCTGGTTTTG CAGCCAAAAT ATCTGCAATG (SEQ ID NO. 107)

Mat alpha signal sequence:

ATGAGATTTC CTTCAATTTT TACTGCAGTT TTATTCGCAG CATCCTCCGC ATTAGCTGCT CCAGTCAACA CTACAACAGA AGATGAAACG GCACAAATTC CGGCTGAAGC TGTCATCGGT TACTTAGATT TAGAAGGGGA TTTCGATGTT GCTGTTTTGC CATTTTCCAA CAGCACAAAT AACGGGTTAT TGTTTATAAA TACTACTATT GCCAGCATTG CTGCTAAAGA AGAAGGGGTA TCTTTGGATA AAAGAGAG (SEQ ID NO. 108)

Extensin signal sequence

CATGGGAAAA ATGGCTTCTC TATTTGCCAC ATTTTTAGTG GTTTTAGTGT CACTTAGCTT AGCTTCTGAA AGCTCAGCAA ATTATCAA (SEQ ID NO.109)

GRP signal sequence

CATGGCTACT ACTAAGCATT TGGCTCTTGC CATCCTTGTC CTCCTTAGCA TTGGTATGAC CACCAGTGCA AGAACCCTCC TA (SEQ ID NO. 110)

On page 56, delete the fourth full paragraph, and replace this paragraph with the following:

The oligonucleotides Asn-T, 5'-A TTC CTG CCA TTC GAG GCG GAA ATC NNG AAC TCG CTG CGT GAT-3' (SEQ ID No. 111) and Asn-B, 5'-ATC ACG CAG CGA GTT CNN GAT TTC CGC CTC GAA TGG CAG GAA T-3' (SEQ ID No. 12), are used in the "quikchange" mutagenesis method by Stratagene (La Jolla, CA) to randomize the first two nucleotides of the Asn 118 codon, AAC. The third base is changed to a G nucleotide, so that reversion to Asn is not possible. In theory a total of 13 different amino acids are created at position 118.